Diverse Regulations of Viral and Host Genes in Tomato Germplasms Responding to Tomato Yellow Leaf Curl Virus Inoculation

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Research Article

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Abstract

Tomato yellow leaf curl virus (TYLCV) is the dominating pathogen of tomato yellow leaf curl disease that caused severe loss to tomato production in China. In this study, we found that a TYLCV-resistant tomato line drastically reduced the accumulation of viral complementary-sense strand mRNAs but just moderately inhibit that of viral DNA and virion-sense strand mRNAs. However, two other resistant lines did not have such virus inhibition pattern. Analysis of differential expressed genes showed that the potential host defense-relevant processes varied in different resistant tomatoes, as compared to the susceptible line, suggesting a diversity of tomato TYLCV-resistance mechanisms.

Main Text

Tomato yellow leaf curl virus (TYLCV) is the dominant species among the more than ten *Bemisia tabaci*-transmitted begomoviruses that cause the tomato yellow leaf curl disease widely occurring in China since 2006 [1-3]. Currently, application of antiviral germplasm is the effective way to control this devastating pathogen in the tomato growing areas and a number of TYLCV-resistant tomato varieties have been developed in China [4,5]. To seek antiviral germplasm befitting the cultivation conditions in southern China, in our preliminary work we screened more than 100 tomato materials from the germplasm bank of Guangzhou Academy of Agricultural Sciences (Guangzhou, China) via resistance identification and obtained three lines (R1, R2 and R3) with different extents of resistance. Both lines R1 and R2 were tested positive by conventional PCR detection at 4 weeks post inoculation (wpi) with the TYLCV infectious clone, and the R1 plants showed leaf curl symptom at 4 wpi but the R2 plants did not until at 6 wpi. Line R3 remained symptomless and negative in PCR detection at 6 wpi. In this study, to investigate the performance of TYLCV in tomato hosts of different resistance levels, we examined the dynamics of TYLCV accumulation and gene expression in the three lines R1~R3, with a susceptible line (S) as the control, which became infected at 2 wpi.

The tomato seeds were germinated on wet towels and sowed in flower pots (9 cm in diameter, each pot with one plant) kept in a growth chamber (25°C, relative humidity 60~70%, 12 h light:12 h dark). When they were in a 4-leaf stage, the seedlings with uniform growth were selected for inoculation of the TYLCV infectious clone, which was kindly offered by Dr. Zifu He from Guangdong Academy of Agricultural Sciences, Guangzhou, China. For each material, thirty plants as three replicates were subject to the inoculation. The viral infectious clone-containing agrobacterium was propagated in LB medium (with 50 µg/mL kanamycin, 10 µg/mL tetracycline and 50 µg/mL rifampicin) under 28°C, 150 rpm shaking for 48 h, resuspended in an inoculation solution (500 µM acetosyringone and 10 mM), and inoculated to the leaf surfaces of the experimental seedlings (2 leaves inoculated for each plants) using a 2-mL syringe. The inoculated plants were kept in the growth chamber under the aforementioned conditions. The leaves of these plants were sampled at 14, 28, and 42 days post inoculation (dpi), respectively, for total DNA/RNA extraction and (RT-)qPCR analyses. The DNA extraction, RNA extraction, qPCR and RT-qPCR were conducted respectively using EasyPure Plant Genomic DNA kit, EasyPure Plant RNA kit, TransStart Tip Green qPCR SuperMix, and TransScript II Green One-Step qRT-PCR SuperMix purchased from TransGen

Biotech Co., Ltd. (Beijing, China), according to the manufacturer's protocols. The RT-qPCR primers reported in the reference [6] were used for the six viral genes (V1, V2, C1~C4), and the same C1 primers were used for qPCR. The ubiquitin (UBI) gene of the host served as an internal control in (RT-)qPCR analyses with primers 5'-TCGTAAGGAGTGCCCTAATGCTGA-3' and 5'-CAATCGCCTCCAGCCTTGTGTAA-3'. In addition, to explore the patterns of TYLCV infection-induced host gene regulation in different tomato lines, an RNA-Seq-based analysis of differential gene expression was conducted. The leaf samples of the lines S, R1, R2 and R3 at 28 dpi were collected (each line with three biological replicates and 10 plants for each replicate), and total RNA extraction and transcriptomic sequencing on the Illumina platform (sequencing mode: paired-end, 2*150bp) were done by Personalbio Technology Co., Ltd. (Nanjing, China) using standard-experiment, quality-control and data-processing procedures.

Neither viral DNA nor mRNAs could be detected by (RT-)qPCR in the plants of line R3 from 14 to 42 dpi. Both lines R1 and R2 became detectable with TYLCV at 28 dpi, but the viral DNA abundances in the two were lower than one half of that in the susceptible line at 28 and 42 dpi (Fig. 1 a). Similar expression patterns were observed for the mRNAs of V1 and V2, the two viral genes on the virion-sense strand (Fig. 1 b and c). However, the expression of the viral mRNAs of C1~C4, the four genes on the complementary-sense strand, was at the drastically low levels in R1 plants but at the moderate levels similar to the expression of V1 and V2 mRNAs in line R2, compared with in the susceptible plants, at 28 dpi and 42 dpi (Fig. 1 d, e, f, g). The begomoviral C1, C2, C3 and C4 proteins involve in viral replication, transcription activation, replication enhancement, and suppressing of RNAi [7-9]. Our findings suggested that tomato resistance to TYLCV may be conferred by different mechanisms in different host lines, including expression inhibition of the viral complementary-sense genes that are required for virus propagation.

The RNA-Seq generated 40.1~41.3 M of clean reads, which consisted of 6.02~6.19 G base pairs (bp), averagely, from the S, R1, R2 and R3 samples, and over 96% of their clean reads were mapped to the tomato chromosomes documented by Solanaceae Genomics Network (<https://solgenomics.net/>). The mapped sequences were annotated to certain functional genes in the GO, KEGG and Swissprot databases, and based on their expression levels normalized by Fragments Per Kilo bases per Million fragments, the differential expressed genes (DEGs) were identified between the resistant and the susceptible lines using the criterion $|\log_2\text{FoldChange}| > 1$ with adjusted p-value < 0.05 . The results showed that compared with the susceptible line, both lines R1 and R2, whose virus resistance lasted relatively shorter, had near-equal numbers of up-regulated and down-regulated genes (949 up-regulated vs. 903 down-regulated ones in R1 plants; 808 up-regulated vs. 793 down-regulated ones in R2 plants); whereas the R3 plants, showing longer resistance, had only 678 up-regulated genes but as many as 1143 down-regulated ones. This quantity difference of DEGs between the hosts of longer and shorter resistance suggested that these two types of anti-TYLCV tomatoes may have distinct host-virus interaction-mediated defense mechanisms. The regulation orientations of DEGs revealed by RNA-Seq were confirmed by RT-qPCR for ten randomly selected DEGs using their sequence-based specific primers and the 28 dpi RNA samples as templates. According to the Swissprot database, 10 up-regulated and 5 down-regulated genes in R1 plants, whereas 4 up-regulated and 10 down-regulated genes in line R3, and only

two down-regulated ones in line R2, were annotated as disease resistance-related genes; and some types of resistance-related DEGs encoding heat shock proteins, Cytochrome P450, E3 ubiquitin-protein ligases, WRKY transcription factors, mitogen-activated protein kinases (which regulate the salicylic acid and jasmonic acid pathways) and auxin signaling proteins were identified in each of the three lines, whereas two lipocalin genes found down regulated in R2 and R3 lines, also suggesting the diversity and complexity of the potential antiviral networks in tomatoes reported previously [10-13]. However, we did not identified the regulation of autophagy-related genes in the three lines, a recently-found mechanism conferring the TYLCV resistance [14].

GO enrichment analysis (Table 1) showed that the DEGs in R1 plants annotated to the top 10 GO terms of significant enrichment (p -value<0.05) largely were associated with DNA binding and transcription (with 146 ones down-regulated), potentially accounting for the low mRNA abundances of the viral genes on the complementary-sense strand found in this study; in R2 plants the DEGs of significant enrichment largely were photosynthesis-related genes (all up-regulated); in R3 plants, most of the DEGs with GO enrichment involved in DNA binding, transcription, and RNA metabolic and biosynthetic processes (largely down-regulated). KEGG enrichment analysis indicated that upon TYLCV infection, the “plant-pathogen interaction” and “phenylpropanoid biosynthesis” pathways commonly in the three resistant lines, the “MAPK signaling” pathway in lines R1 and R2, the “galactose metabolism” pathway in lines R2 and R3, and the “plant hormone signal transduction” pathway in lines R1 and R3, were regulated; whereas each line altered a number of specific pathways responding to the virus infection (Table 1). These findings further suggest different mechanisms potentially underlying host resistance to the same virus in different tomato materials, and confirmed the intricate defensive networks used by the hosts to counteract the virus. In this study, the results of comparative analysis of DEGs between more than one antiviral tomato lines (as well as between lines of longer and shorter resistance) can be a good complement to the previous research on TYLCV-modulated plant transcriptomes using just one resistant and one susceptible host line, or an infected line and an uninfected control [13,15,16]. Further studies may be carried out to explore how the virus-host interactions result in different resistance levels against the virus in various tomato lines.

In summary, we discovered the near-complete expression inhibition of mRNAs of the TYLCV genes on the complementary-sense strand, to our knowledge for the first time, and found different patterns of virus infection-responding gene regulation, which were associated with distinct host defense mechanisms, in tomato lines with different extents of resistance. The findings in this study are helpful to better understand the TYLCV-host interactions relevant to the virus resistance, and provide insight into strategy development of disease resistance-oriented tomato breeding and the control of this economically significant virus.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Data Availability

We declared that the relevant raw data in the manuscript will be freely available to researchers who wish to use them for non-commercial purposes, without breaching participant confidentiality.

Ethical approval

This research did not use human participants or other animals.

Author contributions

DX and JZ conceived the study; WX, XZ and YS performed the experiments; DX, HR and JZ analyzed the data; and DX wrote the manuscript. All authors revised and approved the final manuscript.

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Table

Table 1. The top 10 GO terms and KEGG pathways with significant enrichment of the DEGs in the resistant lines (R1, R2, and R3) respectively relative to the susceptible line

GO/Pathway ID	GO Category*/ KEGG level 1**	GO Term/KEGG level 2	KEGG Pathway	No. of up regulated genes	No. of down regulated genes
<i>GO enrichment analysis</i>					
R1 relative to S					
GO:0140110	MF	transcription regulator activity		15	48
GO:0003700	MF	DNA-binding transcription factor activity		15	41
GO:0006260	BP	DNA replication		13	2
GO:0003677	MF	DNA binding		46	57
GO:0006270	BP	DNA replication initiation		6	0
GO:0019748	BP	secondary metabolic process		15	12
GO:0006952	BP	defense response		15	24
GO:0071554	BP	cell wall organization or biogenesis		23	11
GO:0010383	BP	cell wall polysaccharide metabolic process		5	6
GO:0004553	MF	hydrolase activity, hydrolyzing O-glycosyl compounds		21	8
R2 relative to S					
GO:0031409	MF	pigment binding		21	0
GO:0009768	BP	photosynthesis, light harvesting in photosystem I		21	0
GO:0009765	BP	photosynthesis, light harvesting		21	0
GO:0009522	CC	photosystem I		27	0
GO:0010287	CC	plastoglobule		21	0
GO:0046906	MF	tetrapyrrole binding		46	17
GO:0016168	MF	chlorophyll binding		23	0
GO:0009521	CC	photosystem		30	0

GO:0018298	BP	protein-chromophore linkage		21	0
GO:0009416	BP	response to light stimulus		25	6
R3 relative to S					
GO:0140110	MF	transcription regulator activity		16	64
GO:0003700	MF	DNA-binding transcription factor activity		14	56
GO:0005506	MF	iron ion binding		12	24
GO:0051252	BP	regulation of RNA metabolic process		22	76
GO:1903506	BP	regulation of nucleic acid-templated transcription		21	76
GO:2001141	BP	regulation of RNA biosynthetic process		21	76
GO:0019219	BP	regulation of nucleobase-containing compound metabolic process		22	76
GO:0004497	MF	monooxygenase activity		11	19
GO:0003712	MF	transcription coregulator activity		2	8
GO:0009889	BP	regulation of biosynthetic process		24	76
<i>KEGG enrichment analysis</i>					
R1 relative to S					
sly04626	OS	Environmental adaptation	Plant-pathogen interaction	5	15
sly00480	M	Metabolism of other amino acids	Glutathione metabolism	5	6
sly04075	EIP	Signal transduction	Plant hormone signal transduction	13	11
sly00940	M	Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	14	4
sly03030	GIP	Replication and repair	DNA replication	8	0

sly00340	M	Amino acid metabolism	Histidine metabolism	1	4
sly00330	M	Amino acid metabolism	Arginine and proline metabolism	2	5
sly04016	EIP	Signal transduction	MAPK signaling pathway - plant	6	7
sly00410	M	Metabolism of other amino acids	beta-Alanine metabolism	1	4
sly00500	M	Carbohydrate metabolism	Starch and sucrose metabolism	8	1
sly04626	OS	Environmental adaptation	Plant-pathogen interaction	5	15
R2 relative to S					
sly00196	M	Energy metabolism	Photosynthesis - antenna proteins	22	0
sly00062	M	Lipid metabolism	Fatty acid elongation	4	2
sly00940	M	Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	6	10
sly00941	M	Biosynthesis of other secondary metabolites	Flavonoid biosynthesis	0	7
sly04016	EIP	Signal transduction	MAPK signaling pathway - plant	10	2
sly00520	M	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	6	4
sly00061	M	Lipid metabolism	Fatty acid biosynthesis	2	3
sly00051	M	Carbohydrate metabolism	Fructose and mannose metabolism	4	2
sly00052	M	Carbohydrate metabolism	Galactose metabolism	4	1
sly04626	OS	Environmental adaptation	Plant-pathogen interaction	3	9
R3 relative to S					
sly04626	OS	Environmental adaptation	Plant-pathogen interaction	4	21

sly00330	M	Amino acid metabolism	Arginine and proline metabolism	2	9
sly00340	M	Amino acid metabolism	Histidine metabolism	2	5
sly04141	GIP	Folding, sorting and degradation	Protein processing in endoplasmic reticulum	1	20
sly04075	EIP	Signal transduction	Plant hormone signal transduction	15	13
sly00906	M	Metabolism of terpenoids and polyketides	Carotenoid biosynthesis	2	5
sly00270	M	Amino acid metabolism	Cysteine and methionine metabolism	1	8
sly00052	M	Carbohydrate metabolism	Galactose metabolism	3	3
sly00940	M	Biosynthesis of other secondary metabolites	Phenylpropanoid biosynthesis	6	8
sly04146	CP	Transport and catabolism	Peroxisome	2	4

* BP=biological process; CC=cellular component; MF=molecular function

** CP=cellular processes; EIP=environmental information processing; GIP=genetic information processing; HD=human diseases; M=metabolism; OS=organismal systems

Figures

Figure 1

Abundances of viral DNA (a) and mRNAs of the six viral genes (b~g) relative to the internal control, the host UBI gene (whose abundance is normalized to be 1.0) in the susceptible (S) and three resistant (R1, R2 and R3) tomato lines at 14, 28, and 42 days post inoculation, respectively, of the TYLCV infectious clone. The relative abundances represented by the y-axes were obtained from $2^{-(\Delta Ct)}$.